



White Blood Cell Cryopreservation Protocol

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Blood is collected in purple top tubes containing EDTA as the anti-coagulant. The tubes are centrifuged at 800 x g for 20 min so that the sample is separated into plasma (top layer), buffy coat (middle layer; white blood cells), and erythrocytes (bottom layer; red blood cells). An aliquot of plasma (800µl) is placed in a tube with 200µl of dimethyl sulfoxide and the remainder of the plasma is set aside for use in the next step. The buffy coat is then removed, measured, and diluted to 1 ml with the extra plasma. The buffy coat sample is then diluted with the dimethyl sulfoxide/plasma sample. The remainder of the plasma is then disposed of in accordance with the Hazardous Materials Disposal Protocol of the laboratory. Care should be taken in these first steps to minimize the amount of red blood cells present in the buffy coat samples.

The samples can then be loaded into 0.5ml straws and maintained at 5°C until freezing. Cryopreservation of the white blood cells is performed using a programmable freezer with the following freeze curve: 5°C to -85°C at -3.5°C/minute and then plunge the samples into liquid nitrogen for storage.

Samples are thawed for 30 sec in a 37°C water bath and immediately placed on ice until used or processed further.

References:

Truax, R.E. et al., 1993. Cryopreservation of bovine buffy coat leukocytes for use in immunologic studies. *Am J Vet Res* 54:862-866.

Kleinschuster, S.J. et al., 1979. Cryopreservation of bovine mononuclear leukocytes. *Am J Vet Res* 1649-1651.